

A new stream-dwelling frog of the genus *Litoria* Tschudi, 1838 (Anura: Pelodryadidae) from Salawati Island, Indonesia

urn:lsid:zoobank.org:pub:7B791247-399A-4E15-9DB2-C95357A7EF28

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Abstract: We describe a moderately small (10 males 23.3–26.9 mm, one female 31.5 mm SVL) new species of the speciose pelodryadid genus *Litoria* Tschudi, 1838 from Salawati Island in the Raja Ampat Archipelago, a group of islands off western New Guinea. The new species is mottled green and brown, has extremely long limbs, and was found in lowland tropical rainforest where males called from low vegetation along a rocky, clear-flowing stream. The advertisement call is a series of sharp ‘chip’ notes produced in variable combinations, and sometimes preceded by a longer ‘buzz’ note. Genetically, the new species is related to a clade of predominantly montane, torrent-breeding frogs that is most diverse in the mountains of mainland New Guinea. It appears to be unique among members of this clade in having pigmented eggs. This description brings to three the number of frog species recently described from Salawati that are to date known only from that small land-bridge island.

Key words: Amphibia, treefrog, new species, Raja Ampat Islands, New Guinea.

Introduction

The Raja Ampat islands lie immediately to the west of the Vogelkop Peninsula in West Papua Province, Indonesian New Guinea. The archipelago includes four major islands: Misool, Salawati, Batanta and Waigeo. Unlike Waigeo and Batanta which are oceanic islands with notable bird (Beehler 2007), mammal (Helgen 2007), reptile (Jacobs 2003; Oliver *et al.* 2008) and invertebrate (Polhemus & Allen 2007) endemism, Misool and Salawati are land-bridge islands that share a common shallow water platform with the adjacent mainland (Beehler 2007) and they are generally considered to have a depauperate subset of the nearby mainland fauna (e.g. Helgen 2007). Although the Raja Ampat islands are part of the famous coral triangle that is well known for its significant marine biodiversity (Asaad *et al.* 2018), until recently the

terrestrial herpetofauna of Salawati remained poorly documented. However, recent studies there have resulted in the descriptions of two new species of geckos (*Cyrtodactylus irianjayaensis* Rösler, 2001 and *Lepidodactylus pollostus* Karkkainen, Richards, Kraus, Tjaturadi, Krey *et al.* 2020) (Oliver *et al.* 2008; Karkkainen *et al.* 2020), and two new species of microhylid frogs (*Cophixalus salawatiensis* Günther, Richards, Tjaturadi *et al.* 2015, and *Xenorhina salawati* Günther, Richards, Tjaturadi *et al.* 2020) (Günther *et al.* 2015; 2020). Here we describe a new species of the pelodryadid frog genus *Litoria* from Salawati, bringing to three the number of frogs currently known only from this small land-bridge island. More survey effort on the nearby mainland and on all of the Raja Ampat islands is required before levels of amphibian endemism on Salawati can be assessed with confidence.



Material and methods

Frogs were located using head torches and by tracking advertisement calls. Voucher specimens were fixed in 10% formalin and stored in 70% ethanol. Liver samples for genetic analysis were stored in 90% ethanol prior to specimen fixation. All preserved animals and most genetic samples of the new species are lodged in the Museum Zoologicum Bogoriense, Cibinong, Indonesia (MZB), and several genetic sub-samples are stored in the Australian Biological Tissues Collection (ABTC) at the South Australian Museum, Adelaide, Australia (SAMA). Measurements, terminology, and abbreviations follow Tyler (1968) and Oliver *et al.* (2019). Measurements were made to the nearest 0.1 mm with callipers (SVL - body length from snout to vent, TL - tibia length from heel to outer surface of flexed knee, HL - head length, from tip of snout to posterior margin of tympanum, HW - head width at level of tympana) or a dissecting microscope fitted with an optical micrometer (all other measurements): EN - distance from anterior corner of eye to posterior margin of naris; IN - internarial distance, between medial margins of external nares; EYE - horizontal diameter of eye; TYM - horizontal diameter of tympanum including tympanic annulus; 3FD - transverse diameter of disc of finger 3; 3FP - transverse diameter of penultimate phalanx of finger 3; 4TD - transverse diameter of disc of toe 4 and 4TP - transverse diameter of penultimate phalanx of toe 4. Sex was determined by examination of vocal slits, nuptial pads, the presence of eggs or maturity of oviducts, and by observation of calling. Calls were recorded using a Sennheiser ME66 microphone, K6 powering module, and a Marantz PMD-660 digital recorder. Calls were analysed using Avisoft-SASLab Pro (v4.34, available from Avisoft Bioacoustics <http://www.avisoft.com/>) using procedures and terminology recommended by Köhler *et al.* (2017).

Comparative material was examined at the American Museum of Natural History, New York (AMNH), Natural History Museum, London (BMNH), Museum of Comparative Zoology, Harvard (MCZ), Naturalis, Leiden (RMNH), South Australian Museum, Australia (SAMA), Museum für Naturkunde Berlin (ZMB), the Natural Sciences Resource Centre of the University of Papua New Guinea, Port Moresby (UPNG), Museum Zoologicum Bogoriense, Cibinong, Indonesia (MZB) and the Queensland Museum, Brisbane (QM) (Appendix 1). Additional information for comparisons was taken from published papers on the relevant groups (e.g.

Tyler 1968; Menzies 1993). SJR refers to original field collection numbers of S.J. Richards. Tissues used in the genetic analysis are associated with vouchers in the following additional institutions: Australian Museum, Sydney (AMS), Museum and Art Gallery of the Northern Territory, Darwin (MAGNT), and the Western Australian Museum (WAM).

Frozen or alcohol preserved tissues were available from 36 *Litoria* and *Nyctimystes* specimens representing 23 species, and five outgroup species (Appendix 2). Selection of 'torrent associated' taxa was based on the mitochondrial DNA sequence phylogenetic analysis of Rosauer *et al.* (2009) and a broader unpublished mitochondrial barcode survey of Melanesian pelodyadid frogs (Donnellan, Richards & Mahony unpublished). DNA was extracted using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, U.S.A.) following the manufacturer's protocol for DNA purification from solid tissue. A fragment of the mitochondrial *NADH dehydrogenase subunit 4 (ND4)* gene was amplified and sequenced using the forward primers 5'-TGACTACCAAAAGCTCATGTAGAAGC-3' with the reverse primer 5'-CATTACTTTTACTTGGATTGACCA-3'. Each PCR was carried out in a volume of 25 µl with a final concentration of 1X GeneAmp PCR Gold buffer, 2-4 mM MgCl₂, 200 M of each dNTP, 0.2 mM of each primer and 0.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, U.S.A.). Amplifications consisted of an initial denaturation step of 94 °C for 9 min, followed by 34 cycles of PCR with the following temperature profile: denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min, with an additional final extension at 72 °C for 6 min. The double-stranded amplification products were visualised on 1.5% agarose gels and purified using an UltraClean PCR clean-up DNA purification kit (Mo Bio Laboratories Inc., CA) before cycle-sequencing using the BigDye Terminator v3.1 cycle-sequencing kit (Applied Biosystems). The cycling protocol consisted of 25 cycles of denaturation at 96 °C for 30 s, annealing at 50 °C for 15 s, and extension at 60 °C for 4 min. All samples were sequenced on an Applied Biosystems 3700 DNA sequencer. Sequences were aligned with Muscle v6.814b (Edgar 2004) implemented in Geneious Pro v8.1.4 (Kearse *et al.* 2012) and are deposited in GenBank (Appendix 2).

We used the maximum likelihood approach to reconstruct phylogenetic relationships among the mitochondrial DNA sequences, implemented in IQ-tree (Nguyen *et al.* 2014) on the IQ-TREE webserver (Trifinopoulos *et al.* 2016). We estimated



the best substitution model with ModelFinder (Kalyaanamoorthy *et al.* 2017) following the BIC criterion. We assessed branch support with 1000 ultrafast bootstrap pseudo-replicates (Hoang *et al.* 2017).

Net average sequence divergence between lineages (dA) was calculated in MEGA v7 (Kumar *et al.* 2016) as: $dA = dXY - (dX + dY)/2$, where dXY is the average distance between groups X and Y, and dX and dY are the within-group mean (Table 2).

Results

Description of a new species

The species described herein is assigned to *Litoria sensu* Tyler and Davies (1978) based on its molecular genetic relationships, pending a phylogenetic based resolution of generic boundaries within Pelodryadidae. Our genetic, morphological and acoustic data support the distinctiveness of the new species and indicate that its relationships lie with a clade of *Litoria* from mainland New Guinea that is characterised by its torrent-breeding ecology.

Litoria amnicola sp. nov. (Figs 1–6, 8)

<http://zoobank.org/9E20CE59-18E3-4DDD-8642-5BE7E1276B62>

Suggested English common name: Raja Ampat torrent treefrog.

Holotype adult ♂ MZB Amph.12116 (FN = SJR7728): Weybya Camp, Salawati Island, Raja Ampat Archipelago, West Papua Province, Indonesia (00°57.383'S, 130°47.060'E; 50 m a.s.l.), collected on 24 June 2005 by S. Richards, B. Tjaturadi and K. Krey.

Paratypes 11 specimens: MZB Amph.12099-12104 (FN SJR7738, 7734, 7737, 7758, 7729, 7754); MZB Amph.12106 (SJR7736) MZB Amph.12113-12115, 12117 (SJR 7739, 7759, 7753, 7740), same details as holotype, except MZB Amph.12114 adult female and MZB Amph.12099 subadult female, collected 24-25 June 2005.

Derivatio nominis: From the Latin 'amnis' = a stream of water, and 'cola' = dweller, referring to the preferred habitat of this species.

Diagnosis: A species of *Litoria* that can be distinguished from all congeners by the following combination of characters: 1) size moderately small (10 adult males 23.3-26.9 mm SVL, one adult female 31.5 mm SVL), 2) dorsum finely granular,

mottled green and brown, 3) vomerine teeth barely detectable, 4) ear small, not exceeding 40% of eye diameter, 5) vocal slits present, 6) limbs extremely long (TL/SVL 0.59-0.64), 7) heel without prominent conical tubercle(s), 8) finger webbing not extending beyond tubercle at base of penultimate phalanx on Finger 4, and 9) advertisement call a series of distinctly pulsed, unmusical chirps and buzzes uttered singly or in groups of up to 12 notes.

Description of holotype: Adult male with vocal slits and pale brown nuptial pads. Morphometric data are summarised in Table 1. Body moderately slender, limbs long (TL/SVL 0.61), head moderately wide (HW/SVL 0.34), slightly less than length (HL/SV 0.35, HL/HW 1.05). Vomerine teeth tiny, between and close to edge of choanae; scarcely visible but detectable by scraping with tip of forceps. Tongue broadly oval with distinct, broad posterior notch. Vocal slits long, extending from slightly anterior of angle of jaws halfway to front of mouth. Snout broadly rounded with tip slightly truncated in dorsal view (Fig. 1), truncate (nearly vertical) in lateral view. Canthus rostralis well defined, curved; loreal region steep, concave; nostrils closer to tip of snout than to eyes; internarial distance greater than distance from external naris to eye (EN/IN 0.85, IN/SVL 0.11, EN/SVL 0.09); eyes large (EYE/SVL 0.14), prominent, protruding in dorsal and ventral views; pupil horizontal, without pigment flecks on nictitating membrane. Tympanum small (TYM/SVL 0.05), slightly less than one third diameter of eye (TYM/EYE = 0.32), annulus distinct except dorsal third obscured by thick, slightly curved supratympanic ridge; series of small, pale tubercles located below tympanum. Skin of dorsum finely granular, less so (nearly smooth) on dorsal surfaces of limbs; ventrally throat and chest smooth with numerous scattered low tubercles; abdomen coarsely granular (Figs 1, 3). Ventral surfaces of limbs smooth. A patch of large, white tubercles below vent extends distally approximately 4 mm along posterior margins of each thigh. A series of low, pale tubercles along outer edge of foot, extending from heel to midpoint of T5. Some scattered pale tubercles on outer edge of forearm, not forming linear ridge. Fingers moderately short with expanded terminal discs (3FD/SVL 0.05) with distinct marginal grooves; relative lengths of fingers: 3>4>2>1; webbing limited, on inside of F4 reaching to just below subarticular tubercle at base of penultimate phalanx, continuing as broad flange along edge of finger to base of disc; on outside of F3 reaching only halfway to tubercle at base of penultimate phalanx then continuing to



disc as broad flange (Fig. 2), webbing only a basal trace between 2 and 3, absent between 1 and 2. Hand with poorly defined narrow inner, and oval outer, metacarpal tubercles, and distinct narrow dermal ridge along outer edge of hand. Nuptial pad on first finger pale brown, elongate (1.6 mm long), broader distally (0.5 mm) than proximally (0.25 mm). Toes with expanded terminal discs with terminal grooves, 4TD same as 3FD (3FD/4TD 1.00). Webbing between toes reaches to base of disc on inside of T5, and on outside of T3 and T2; and to about halfway to subarticular tubercle at base of penultimate phalanx on both sides of T4, then extending to disc as narrow flange; relative lengths of toes: $4 > 5 > 3 > 2 > 1$, but T5 only marginally longer than T3. Foot with narrow, elongate inner, and small, low, round outer metatarsal tubercle.

Colouration in life (Fig. 5): body and limbs rufous brown dorsally, extensively mottled with bright green; brown areas most intense on anterior of mid-dorsum, on snout including loreal region, and in broad band across dorsal surfaces of both thighs, these brown patches enclosing more intense green blotches. Green patch between eyes forming poorly defined inter-orbital bar. Posterolateral surfaces of body translucent, with scattered low, pale-cream tubercles; prominent pale-cream tubercles extend in short row from posterior edge of eye below tympanum to posterior edge of curved supratympanic ridge. Ventrally abdomen white, throat yellowy cream without dark stippling around angle of jaws; ventrolateral surfaces of body and ventral surfaces of thighs and tibiae translucent pinkish-grey, tibiae bones visible through skin (Fig. 3); posterior surfaces of thighs uniform grey and hidden surfaces of thighs peppered with brown spots. Peppering of fine dark-brown specks present on dorsal surfaces of limbs, extending onto dorsal surfaces of fingers, toes and webbing. A row of low, pale-cream tubercles along outer edge of foot and several small pale-cream tubercles on outer edge of forearm. Iris pale yellow with rufous brown reticulations.

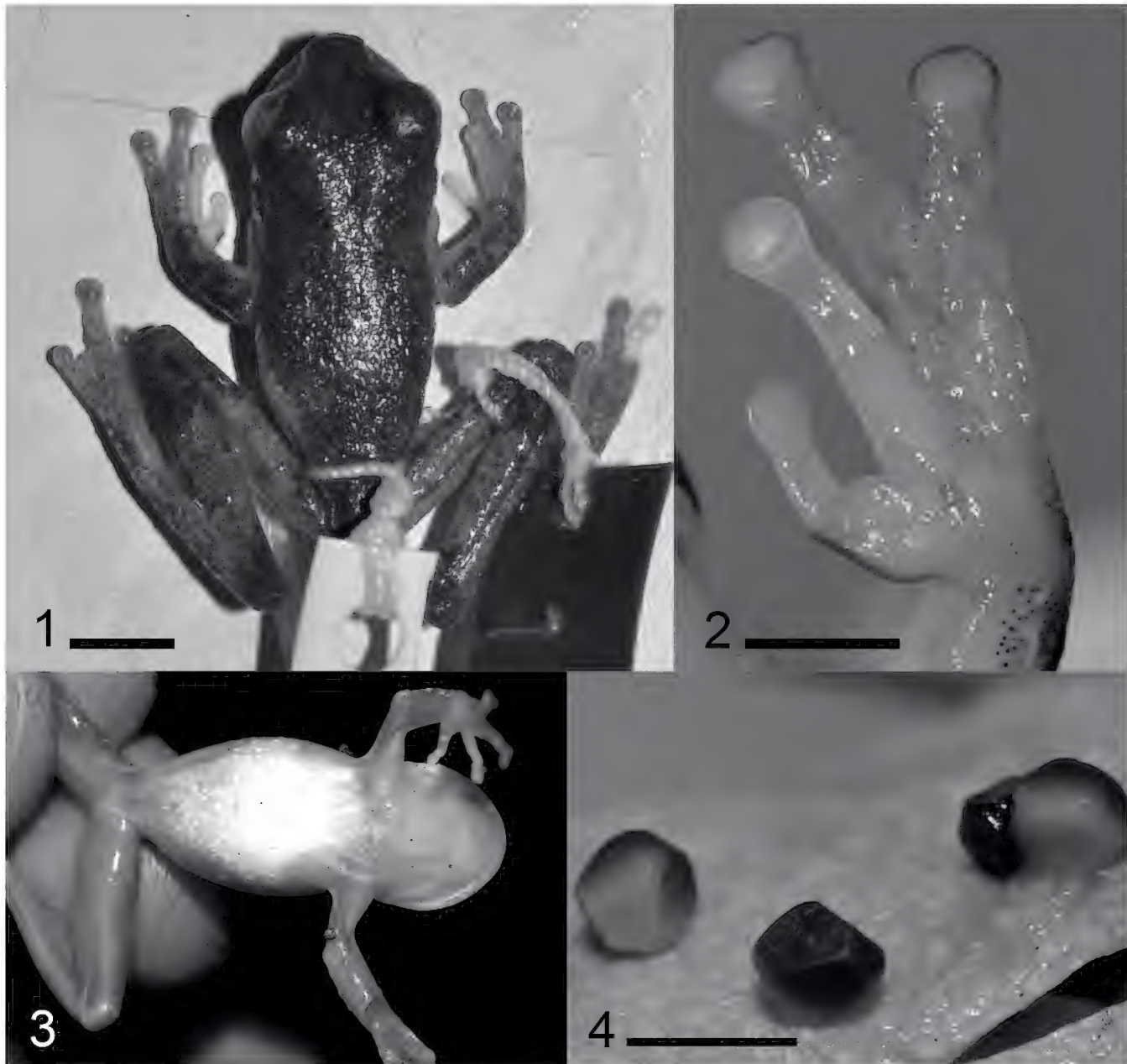
Colouration in preservative: green markings on dorsum and limbs have become blue, background brown and fine brown peppering have become purplish brown; fine brown peppering on limbs more conspicuous in preservative than in life. Ventral surfaces uniformly cream, except for extensive fine brown peppering on plantar surfaces; peppering restricted to few scattered spots on palmar surfaces.

Variation in the type series: Measurements and body proportions of the type series show

limited variation (Table 1). Male body size 23.3–26.9 mm SVL; one adult female (MZB Amph.12114) with SVL of 31.5 mm, one immature female (MZB Amph.12099) with SVL of 27.3 mm. Snout normally broadly rounded with truncate tip in dorsal view, but MZB Amph.12102 with more pointed tip. Vomerine teeth normally detectable only by scraping with forceps, but visible (though very small) in several specimens including MZB Amph.12114 (adult female). 3FD is larger than 4TD in all but three of the types, in which $3FD = 4TD$. In preservative dorsal colour variable, ranges from pale (e.g. MZB Amph.12115) to dark (MZB Amph.12113) brown; extent of blue markings is highly variable, from few, small, scattered spots (e.g. MZB Amph.12104) to extensive blue patches across much of dorsum (e.g. MZB Amph.12113). Fine dark brown stippling on body and limbs more conspicuous on paler specimens and on lower jaw. Most specimens with narrow blue (green in life) bar between eyes or immediately anterior of eyes; this bar complete or broken into series of spots (e.g. MZB Amph.12114). Row of pale tubercles below tympanum invariably present but varies from scarcely detectable to prominent (see e.g. fig. 6).

Advertisement call: Our analysis of the call structure of *Litoria amnicola* sp. nov. is based on detailed examination of 11 calls produced by the holotype (MZB Amph.12116) at an air temperature of 26 °C. Calls of *L. amnicola* sp. nov. are series of 2–13 short, distinctly pulsed notes sounding to the ear like a harsh ‘chip’, produced singly ‘single’, in couplets ‘double’ notes, or (rarely) in triplets ‘triple’ notes, occasionally preceded by one or two longer ‘buzz’ notes (Fig. 8). Because the sequence and rate at which ‘single’, ‘double’, and ‘triple’ notes are produced is variable we defined groups of notes as separate calls if the first note in one group was separated from the last note in the previous group by at least two seconds, irrespective of the note type. Eleven calls of the holotype were produced at a rate of 0.15 calls/s, with intervals of 2.95–7.02 s between calls (mean = 4.49; SD = 1.38). Dominant frequency is 2858–3081 Hz (mean = 3004, SD = 85.80). Of the eleven calls analysed, four contain one ‘buzz’ note and one contains two consecutive ‘buzz’ notes; ‘buzz’ notes always precede ‘chip’ notes. ‘Buzz’ notes are 0.13–0.19 s long (mean = 0.16, SD = 0.02) and contain 18–25 pulses (mean = 21.7, SD = 2.5), produced at a rate of 126–133 pulses/s (mean = 129.7, SD = 2.29, n = 6). In contrast, 81 shorter ‘chip’ notes are just 0.007–0.046 s long (mean = 0.021, SD = 0.008) and contain 2–7 pulses (mean = 3.6, SD





Figures 1-4. *Litoria amnicola* sp. nov. 1 – Dorsal view of preserved holotype (MZB Amph.12116) [scale bar = 5 mm]; 2 – Left hand of holotype (MZB Amph.12116) in palmar view [scale bar = 2 mm]; 3 – Ventral view of holotype (MZB Amph.12116) in life - note visibility of bones through skin; 4 – Three pigmented eggs extracted from adult female paratype (MZB Amph.12114) [scale bar = 2 mm].

= 0.94), produced at a rate of 130-214 pulses/s (mean = 154.7, SD = 19.01, n = 81). Twenty-one of the ‘chip’ notes analysed (26%) are produced as ‘single’ notes, 54 (67%) are produced as ‘double notes’ (Fig. 8), and six (7%) are produced as triple notes. The substantial differences in both length and pulse number between ‘buzz’ and ‘chip’ notes suggest that they do not represent ends of a spectrum but are discrete note types.

Differential diagnosis: *Litoria amnicola* sp. nov. is compared here with small (male SVL ~20-30 mm), green or green and brown New Guinean *Litoria* that do not breed in lotic waterbodies or for which breeding biology is unknown, as follows:

Litoria amnicola sp. nov. differs from *L. albolabris* (Wandolleck, 1911) and *L. mystax* (Van Kampen, 1906) in having dorsum brown and green (vs uniform green), dorsum granular (vs smooth), in having a series of pale cream tubercles beneath the tympanum (vs a single white bar), and limbs longer (TL/SVL 0.59-0.64 vs 0.50~0.55 in *L. albolabris* and 0.54 in *L. mystax*; Tyler 1968); from members of the *L. bicolor* (Gray, 1842) group (*L. bibonius* Kraus et Allison, 2004, *L. chloristona* Menzies, Richards et Tyler, 2008, *L. contrastens* (Tyler, 1968), *L. euryrnastes* Menzies, Richards et Tyler, 2008, *L. lodesdema* Menzies, Richards et Tyler, 2008, and *L. viranula* Menzies, Richards et Tyler, 2008),





Figures 5-7. *Litoria amnicola* sp. nov. 5 – Holotype (MZB Amph.12116) in life; 6 – Paratype (MZB Amph.12115) in life; 7 – Habitat of *Litoria amnicola* sp. nov. at the type locality. Males called from low vegetation adjacent to this stream.

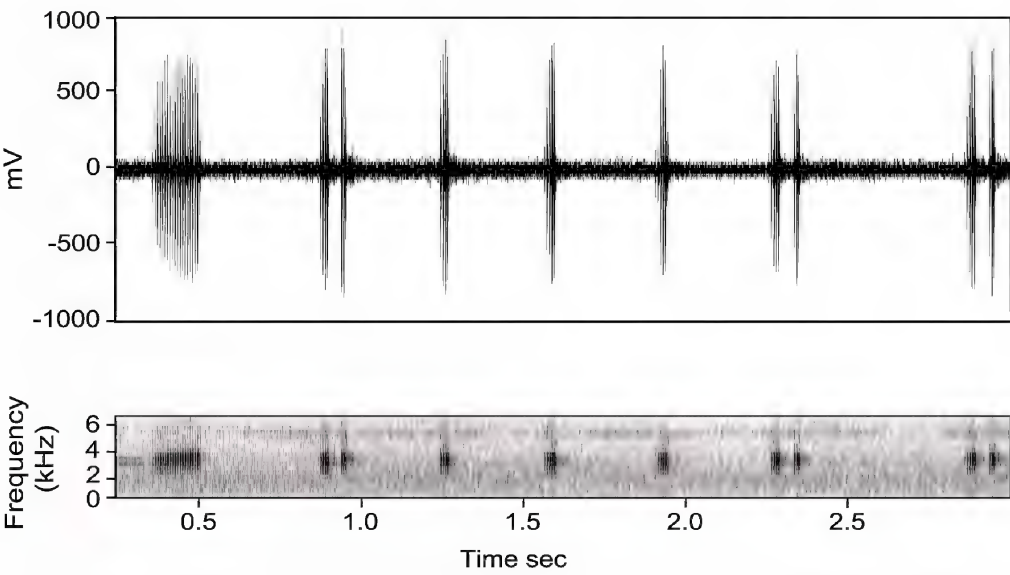


Figure 8. Waveform (top) and spectrogram (bottom) of an advertisement call of *Litoria amnicola* sp. nov. (holotype: MZB Amph.12116) showing variation in note structure including an introductory 'buzz' note and nine 'chip' notes produced individually and in couplets.



in having dorsum brown mottled with green (vs predominantly green or occasionally brown), tarsi with row of tubercles on outer margins (vs absent), and snout truncate in lateral view and at tip in dorsal view (vs rounded in lateral view and obtusely pointed or rounded in lateral view; Kraus & Allison (2004a); Menzies *et al.* (2008)); from *L. christianbergmanni* Günther, 2008 in its smaller size (male SVL 26.9–31.2 mm in *L. christianbergmanni*), dorsum brown and green (vs uniform green with white, yellow or pale green spots), and white bar below eye lacking (vs present in *L. christianbergmanni*); from *L. chloronota* (Boulenger, 1911) in having body size smaller (males 27–32 mm in *L. chloronota*), dorsum brown and green (vs mottled pale and darker green with or without yellow spots), snout broader (EN/IN 0.79–0.97 vs 0.63–0.71 in *L. chloronota*), and limbs longer (TL/SVL 0.59–0.64 vs 0.52–0.58 in *L. chloronota*; Menzies 1993); from members of the *L. gracilentia* (Peters, 1869) group (*L. aruensis* (Horst, 1883), *L. auae* Menzies *et al.*, 2004, *L. callista* Kraus, 2013, *L. elkeae* Günther *et al.*, 2000, *L. eschata* Kraus *et al.*, 2009, *L. kumae* Menzies *et al.*, 2004, and *L. robinsonae* Oliver, Stuart-Fox *et al.*, 2008) in having dorsum brown and green (vs plain green with or without pale or dark spots), and pale canthal and postocular stripes absent (vs present: Menzies and Tyler 2004; Kraus 2013); from *L. havina* Menzies, 1993 in having dorsum brown and green (vs uniformly green), and fleshy rostral spike in males lacking (vs present); from *L. rubrops* Kraus *et al.*, 2004 in having dorsum brown and green (vs green, usually speckled with black or darker green), vomerine teeth small but detectable (vs absent in *L. rubrops*), limbs longer (TL/SVL 0.59–0.64 vs 0.48–0.59 in *L. rubrops*), and iris pale yellow with rufous brown reticulations (vs iris red in *L. rubrops*; Kraus & Allison 2004b); from *L. nigropunctata* (Meyer, 1875), *L. iris* (Tyler, 1962), *L. majikthise* Johnston *et al.*, 1994, *L. ollauro* Menzies, 1993, *L. richardsi* Dennis *et al.*, 2006, *L. singadanae* Richards, 2005, and *L. verae* Günther, 2004, in having fingers with limited webbing (not extending beyond subarticular tubercle at base of penultimate phalanx, vs finger webbing extensive, extending beyond subarticular tubercle at base of penultimate phalanx), and further from *L. iris* in lacking (vs having) violet patch in axilla and having posterior surfaces of thighs uniform grey (vs posterior of thighs blue, red, or yellow, frequently blotched with white or purple, in *L. iris*), from *L. ollauro* in having dorsum variably green and brown (vs uniform green or green with yellow spots in

L. ollauro) (Menzies 1993), from *L. majikthise* by having posterior surfaces of thighs uniform grey in life (vs red in *L. majikthise*), and by pearl-white postocular bar lacking (present in *L. majikthise*), from *L. richardsi* in having dorsum mottled brown and green (vs dorsum with irregular black lines), throat and finger and toe webbing without extensive black markings (vs present in *L. richardsi*), and periphery of tympanic membrane not transparent (vs transparent in *L. richardsi*), from *L. singadanae* in having posterolateral surfaces of belly and posterior surfaces of thighs uniform translucent pinkish grey in life (vs posterior of venter and hidden surfaces of legs uniform orange in *L. singadanae*), tympanum much smaller (TYM/EYE 0.25–0.40 vs 0.69–0.81 in *L. singadanae*), tympanic membrane pigmented (vs transparent in *L. singadanae*), and from *L. verae* in prominent crenulated skin fold along outer margins of tarsi lacking (vs present), and dorsum without small brown spots aligned transversely (vs present in *L. verae*).

Litoria amnicola sp. nov. is compared here with small (male SVL ~20–30 mm) green, green and brown or predominantly brown New Guinean *Litoria* that breed in lotic waterbodies or that are presumed to do so, as follows: *Litoria amnicola* sp. nov. differs from *L. bulmeri* (Tyler, 1968) by size smaller (males 23.3–26.9 mm vs 29–34 mm SVL), dorsum brown and green (vs uniform green) and black lateral stripe absent (vs present); from *L. brongersmai* by having legs longer (TL/SVL 0.59–0.64 vs 0.52–0.54) snout truncate (vs rounded) and dorsum brown and green (vs dark brown) (Tyler 1968); from *L. dorsivena* (Tyler, 1968) by canthus rostralis curved (vs straight), vomerine teeth poorly developed (vs prominent) and dorsum brown and green (vs brown) (Tyler 1968); from *L. fuscata* Oliver *et al.*, 2007, by size smaller (males 23.3–26.9 mm vs 29–30 mm SVL), legs longer (TL/SVL 0.59–0.64 vs 0.52–0.54) and having dorsum brown and green (vs uniform brown) (Oliver & Richards 2007); from *L. longicrus* (Boulenger, 1911) in having dorsum brown and green (vs green), dorsum granular (vs smooth), and in having a series of pale cream tubercles beneath the tympanum (vs a single white bar); from *L. modica* (Tyler, 1968) in having snout tip truncate at tip (vs rounded in both dorsal and lateral views), skin of dorsum granular (vs smooth with scattered low tubercles), tongue with posterior notch (vs absent), and eggs unpigmented (vs pigmented); from *L. napaea* (Tyler, 1968) by size larger (males 23.3–26.9 mm vs 18.8–22.7 mm SVL), skin granular (vs smooth), snout truncate (vs obtusely pointed) and eggs unpigmented (vs



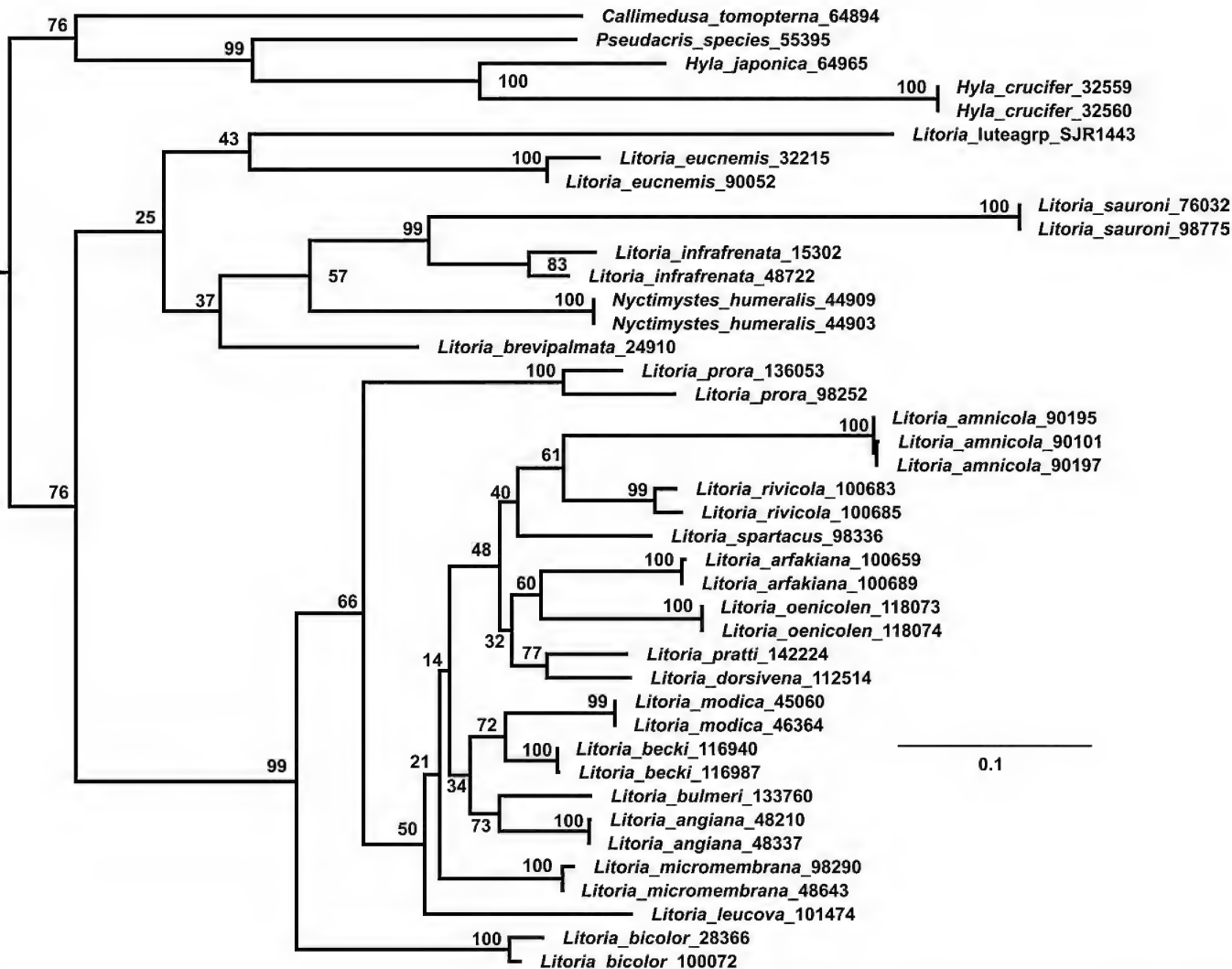


Figure 9. Maximum likelihood (ML) phylogram of relationships between mitochondrial ND4 nucleotide sequences of *Litoria* species. Numbers at nodes are ML bootstrap proportions.

pigmented) (Tyler 1968); from *L. pratti* (Boulenger, 1911) by size smaller (males 23.3-26.9 mm vs 29.5-31.5 mm SVL) (Tyler 1968), canthus rostralis curved (vs straight) and vomerine teeth poorly developed (vs well developed); from *L. rara* Günther et Richards, 2005 by size larger (males 23.3-26.9 mm vs 20.4 mm SVL), skin dorsally finely granular (vs smooth), and dorsum brown mottled with green (vs dark grey); and from *L. scabra* Günther et Richards, 2005 in having skin of dorsum finely granular (vs strongly tubercular), crenulated dermal ridge on foot and forearm absent (vs present), and snout truncate in lateral view (vs acute, projecting beyond lower jaw). From the new species' closest known relative, *Litoria rivicola* Günther et Richards, 2005 (see *Molecular genetic comparisons* below), *Litoria amnicola* sp. nov. can be distinguished by size larger (males 23.3-26.9 mm vs 20.4-22.1 mm SVL), dorsum finely granular (vs coarsely tubercular), and snout truncate (vs acutely projecting) (Günther & Richards 2005).

Distribution and habitat: *Litoria amnicola*

sp. nov. is a scansorial species that is currently known only from a single location at an altitude of about 50 m a.s.l. near the north coast of Salawati Island (Map 1), where males called from heights of between 50 cm and 3 m on low foliage along a clear-flowing, rocky stream in lowland rainforest (Fig. 7). None were observed adjacent to forest pools or other lentic habitats. It is not known whether *Litoria amnicola* sp. nov. is more widespread on Salawati. The type locality is at the northern edge of the only substantial mountain range on the island, and it is possible that the species' distribution there is constrained by the availability of cool, fast-flowing streams draining the mountains. Images of a small treefrog taken recently along a similar clear-flowing stream on Batanta Island by Dr. Tibor Kovács may represent this species, but voucher material is required to confirm that record. On Salawati, calling males climbed onto shrubs and started calling before dark, and calling activity continued until after midnight each night. An adult female (MZB Amph.12114) contained heavily pigmented



eggs; three eggs extracted for examination (Fig. 4) measured 1.3–1.5 mm diameter (mean = 1.4 mm). Although the deposition of pigmented eggs in forest streams is common among some groups of Melanesian pelodryadid frogs (e.g. the *Litoria genimaculata* (Horst, 1883) group), this is the first reported case of a species within the large clade of montane, torrent-breeding *Litoria* from New Guinea (Fig. 9) that is known to have pigmented ova (e.g. Tyler 1968; Tyler & Davies 1978; Menzies 2006; S.J. Richards, unpublished data). The eggs of the closest known relative of *L. amincola* sp. nov., *L. rivicola*, have not been reported but tadpoles have a large, ventrally located oral disc with numerous papillae, as is typical of other torrent-breeding species (Günther & Richards 2005). The tadpole of *L. amincola* sp. nov. has yet to be discovered. The eggs and tadpoles of many New Guinean torrent-breeding *Litoria* have not been documented, and it is possible that additional species within this clade have pigmented ova. Integrated reproductive, ecological and molecular studies are required to provide a better understanding of the evolution of reproductive traits in this group of frogs.

Molecular genetic comparisons: The final alignment for the mitochondrial *ND4* gene comprised 845 bp. In a phylogram of relationships among mitochondrial *ND4* sequences, the sequences from *L. amincola* sp. nov. are the sister to the two *L. rivicola* sequences with strong support and both sit within the “torrent-breeding frog” group (Fig. 9). The net uncorrected sequence divergence (*dA*) for *ND4* between *L. amincola* sp. nov. and its sister taxon, *L. rivicola*, was 0.14, which is at the upper end of the range of values among other sister taxon pair comparisons within the torrent-breeding frog group (0.11–0.15, Table 2).

Acknowledgements

Fieldwork in the Raja Ampat Islands was supported by Conservation International and the South Australian Museum. We are most grateful to Yance de Fretes, Muhamad Farid and Jatna Supriatna of Conservation International for their organisational skills and support, and to Herlina Kafiari, Rizana Kurniati, Elias Kore, Sofia Roni, Arthur Tipawael and Adelina Werimon for assistance in the field. We are also extremely grateful to the Indonesian Institute of Sciences (LIPI) for their support and approval of specimen export, the Forestry Department, especially Kantor Sub BKSDA 2, Sorong and Directorate Jenderal PHKA, and to

Lisa Capon who produced Figs 1–8 and the map, and Tibor Kovács who kindly provided images of a similar *Litoria* species from Batanta Island while this manuscript was in review. Linda Ford (then AMNH), Barry Clarke (BMNH), José Rosado (MCZ), Hellen Kurniati and Mumpuni (MZB), Pim Arntzen (RMNH), Carolyn Kovach and Mark Hutchinson (SAMA), and Rainer Günther (ZMB) kindly provided access to specimens in their care, and tissue samples were provided by Paul Doughty, Rainer Günther and Michael Mahony. We also thank Luke Price, Steven Myers and Tessa Bradford for performing the DNA sequencing, and Paul Oliver and Rainer Günther for their careful and constructive reviews of the manuscript.

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- Received: 31.viii.2020.
Accepted: 15.xi.2020.

Table 1. Measurements (mm) and ratios of the type series of *Litoria amnicola* sp. nov.

All vouchers have MZB Amph registration numbers. *MZB Amph.12116 is the holotype. All specimens are adult males except MZB Amph.12114 which is an adult female and MZB Amph.12099 which is a subadult female. Means, ranges and SD calculated from males only.

MZB#	12100	12101	12102	12103	12104	12106	12113	12115	12116*	12117	12099	12114	Mean	Range	SD
SVL	26.9	25.4	26.3	26.6	23.3	23.7	25.5	25.3	24.3	23.9	27.3	31.5	25.12	23.3-26.9	1.27
TL	16	15.8	15.9	16.2	14.6	14.7	15.5	15.6	14.8	15.4	16.3	19.1	15.45	14.6-16.2	0.57
HW	9.2	8.6	8.9	8.9	8.3	7.9	8.2	9	8.2	8.6	8.9	10.2	8.58	7.9-9.2	0.42
HL	9.3	9.6	9.7	9.4	8.6	8.7	9	9.2	8.6	8.9	9.9	11	9.1	8.6-9.7	0.41
EYE	3.3	3.6	3.1	3.6	3	3.5	3.5	3.5	3.4	3.4	3.2	3.5	3.39	3.0-3.6	0.2
TYM	1.2	1.2	1.1	0.9	1.2	1	1.2	0.9	1.1	1.2	1.2	1.4	1.1	0.9-1.2	0.12
EN	2.4	2.7	2.8	2.6	2.5	2.4	2.5	2.5	2.3	2.3	2.9	3.2	2.5	2.3-2.8	0.16
IN	3	2.9	2.9	2.9	2.6	2.8	2.8	2.9	2.7	2.9	3.1	3.3	2.84	2.6-3.0	0.12
3FD	1.4	1.5	1.5	1.5	1.2	1.5	1.5	1.4	1.3	1.3	1.4	1.8	1.41	1.2-1.5	0.11
3FP	0.9	0.9	1	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8	1	0.88	0.8-1.0	0.06
4TD	1.2	1.3	1.3	1.4	1.2	1.4	1.4	1.3	1.3	1.3	1.3	1.6	1.31	1.2-1.4	0.07
4TP	0.9	1	1.1	1	0.8	1	0.9	1	0.9	1	0.9	1.1	0.96	0.8-1.1	0.08
HL/SVL	0.35	0.38	0.37	0.35	0.37	0.37	0.35	0.36	0.35	0.37	0.36	0.35	0.36	0.35-0.38	0.01
HW/SVL	0.34	0.34	0.34	0.33	0.36	0.33	0.32	0.36	0.34	0.36	0.33	0.32	0.34	0.32-0.36	0.01
HL/HW	1.01	1.12	1.09	1.06	1.04	1.1	1.1	1.02	1.05	1.03	1.11	1.08	1.06	1.01-1.12	0.04
EYE/SVL	0.12	0.14	0.12	0.14	0.13	0.15	0.14	0.14	0.14	0.14	0.12	0.11	0.14	0.12-0.15	0.01
TYM/EYE	0.36	0.33	0.35	0.25	0.4	0.29	0.34	0.26	0.32	0.35	0.38	0.4	0.33	0.25-0.40	0.05
TYM/SVL	0.04	0.05	0.04	0.03	0.05	0.04	0.05	0.04	0.05	0.05	0.04	0.04	0.04	0.03-0.05	0.01
TL/SVL	0.59	0.62	0.6	0.61	0.63	0.62	0.61	0.62	0.61	0.64	0.6	0.61	0.62	0.59-0.64	0.01
EN/IN	0.8	0.93	0.97	0.9	0.96	0.86	0.89	0.86	0.85	0.79	0.94	0.97	0.88	0.79-0.97	0.06
IN/SVL	0.11	0.11	0.11	0.11	0.11	0.12	0.11	0.11	0.11	0.12	0.11	0.1	0.11	0.11-0.12	0
EN/SVL	0.09	0.11	0.11	0.1	0.11	0.1	0.1	0.1	0.09	0.1	0.11	0.1	0.1	0.09-0.11	0.01
4TD/4TP	1.33	1.3	1.18	1.4	1.5	1.4	1.56	1.3	1.44	1.3	1.44	1.45	1.37	1.18-1.56	0.11
4TD/SVL	0.04	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.04-0.06	0
3FD/SVL	0.05	0.06	0.06	0.06	0.05	0.06	0.06	0.06	0.05	0.05	0.05	0.06	0.06	0.05-0.06	0
3FD/3FP	1.56	1.67	1.5	1.67	1.5	1.67	1.88	1.56	1.63	1.44	1.75	1.8	1.61	1.44-1.88	0.12
3FD/4TD	1.17	1.15	1.15	1.07	1	1.07	1.07	1.08	1	1	1.08	1.13	1.08	1.00-1.17	0.06



Table 2. Net average sequence divergence (dA) for the mitochondrial *ND4* gene between sister species pairs of *Litoria* from the “torrent-breeding” frog group.

Sister species pair	dA
<i>Litoria rivicola</i> – <i>L. amnicola</i> sp. nov.	0.14
<i>L. angiana</i> – <i>L. bulmeri</i>	0.12
<i>L. arfakiana</i> – <i>L. oenicolon</i>	0.15
<i>L. becki</i> – <i>L. modica</i>	0.11
<i>L. dorsivena</i> – <i>L. pratti</i>	0.11

Appendix 1. Specimens examined.

***Litoria albolabris* (Wandolleck, 1911)**

Papua New Guinea: Sandaun Province, Aitape, SAMA R4947 (syntype).

***Litoria auae* Menzies et Tyler, 2004**

Papua New Guinea: Gulf Province, Purari River, near McDowell Is, UP2490 (holotype), SAMA R57262-63 (paratypes).

***Litoria bulmeri* (Tyler, 1968)**

Papua New Guinea: Madang Province, Upper Aunjung Valley, Schrader Mtns, SAMA R5625 (holotype).

***Litoria chloristona* Menzies, Richards et Tyler, 2008**

Papua New Guinea: National Capital District, Waigani, SAMA R9122a; Central Province, Brown River, SAMA R13251a-c; Gulf Province, Kopi, SAMA R63502-9 (all paratypes).

***Litoria chloronota* (Boulenger, 1911)**

Indonesia: West Papua Province, Arfak Mountains, BM1947.2.31.20 (syntype), UP8380-8.

***Litoria contrastens* (Tyler, 1968)**

Papua New Guinea: Eastern Highlands Province, Barabuna, SAMA R5845 (holotype), SAMA R6450 (paratype); Western Highlands Province, Noreikova, SAMA R5847 (5 specimens).

***Litoria dorsivena* (Tyler, 1968)**

Papua New Guinea: West Sepik Province, Telefomin, SAMA R7901 (holotype).

***Litoria elkeae* Günther et Richards, 2000**

Indonesia: Papua Province, Siewa River, MZB Amph.3866-9, QMJ70490-2 (all paratypes).

***Litoria euryrnastes* Menzies, Richards et Tyler, 2008**

Indonesia: Papua Province, Siewa River, MZB Amph.14651-5; Papua New Guinea: Manus

Province, Lorengau, SAMA R63497-501 (all paratypes).

***Litoria fuscula* Oliver et Richards, 2007**

Indonesia: Papua Province, Derewo River Basin, MZB Amph.11822 (holotype), SAMA R60724 (paratype).

***Litoria havina* Menzies, 1993**

Papua New Guinea: Western Province, Ok Kam, UP7281 (holotype); Western Province, Ok Ma, UP 8406-7 (paratypes); Western Province, Ok Kam, SAMA R38596-7; Southern Highlands Province, Agogo Range, SAMA R60173-7.

***Litoria iris* (Tyler, 1963)**

Papua New Guinea: Chimbu Province, Bamna, BM 1961.1226 (holotype); Southern Highlands Province, Tari, UP3115-35; Eastern Highlands Province, Ubaigubi, UP8289-90; Enga Province, Porgera, UP 7148-67, Lake Tawa, SAMA R71599; Sandaun Province, Telefomin, SAMA R5423, R5874; Hela Province, Gigira Ridge, SAMA R71598.

***Litoria kumae* Menzies et Tyler, 2004**

Papua New Guinea: Southern Highlands Province, Tari, UP3108 (holotype), SAMA R52760-61 (paratypes).

***Litoria leucova* (Tyler, 1968)**

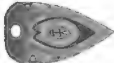
Papua New Guinea: Sandaun Province, Mt Stolle, SAMA R44091-2, UP8604-6.

***Litoria lodesdema* Menzies, Richards et Tyler, 2008**

Papua New Guinea: East New Britain Province, Kerevat, SAMAR7046-47, R7049, R7055-56, R7058-59, R8439a,b.

***Litoria longicrus* (Boulenger, 1911)**

Indonesia: Papua Province, Wendessi, BM 1947.2.22.60-61 (syntypes).



***Litoria majikthise* Johnston et Richards, 1994**

Papua New Guinea: Western Province, Tabubil, SAMA R44093 (holotype), UP6734, 7305-9, 8501-8, 8602-3, SAMA R44094-44101 (all paratypes).

***Litoria modica* (Tyler, 1968)**

Papua New Guinea: Eastern Highlands Province, Oruge, MCZ 52856 (holotype), MCZ 52857-52861, SAMA R8108 (paratypes).

***Litoria mucro* Menzies, 1993**

Papua New Guinea: East Sepik Province, Near Rauti Village, UP2741-3, 2745-56 (paratypes).

***Litoria mystax* (van Kampen, 1906)**

Indonesia: Papua Province, Moaif, RMNH 4632 (holotype).

***Litoria napeae* (Tyler, 1968)**

Indonesia: Papua Province, Snow Mountains, Bernhard Camp, AMNH 49573, 49575, 49657, 77022-24 (paratypes).

***Litoria nigropunctata* (Meyer, 1875)**

Indonesia: Papua Province, Yapen Island, Mount Waira, ZMB 63977, Yapen Island near Konti, SAMA R61799.

***Litoria ollauro* Menzies, 1993**

Papua New Guinea: Milne Bay Province, Agaun, UP4644 (holotype), UP4634-43, 4645-51, 5070-89 (paratypes).

***Litoria pratti* (Boulenger, 1911)**

Indonesia: Papua Province, Wendessi, BMNH 1947.2.23.54-56, Arfak Mountains, BMNH 1947.2.23.57-58 (syntypes).

***Litoria richardsi* Dennis et Cunningham, 2006**

Papua New Guinea: Western Province, Tabubil, SAMA R60283 (holotype); Upper Fly River, SAMA R71602-5; Indonesia: Papua Province, Tiri River, Mamberamo Drainage, MZB Amph.11823 (paratype).

***Litoria robinsonae* Oliver, Stuart-Fox et Richards, 2008**

Papua New Guinea: Gulf Province, Ivimka, Lakekamu Basin, SAMA R55527 (holotype), R55528-9 (paratypes).

***Litoria scabra* Günther et Richards, 2005**

Indonesia: Papua Province, Wapoga River Drainage, MZB Amph.11335 (holotype), Amph.11336-11340, SAMA R60707-9, ZMB67357-9 (paratypes).

***Litoria singadanae* Richards, 2005**

Papua New Guinea: Morobe Province, Ridge above Surim Camp, eastern Finisterre Mountains, SAMA R60172 (holotype), UP 9968, SAMA R60171 (paratypes).

***Litoria verae* Günther, 2004**

Indonesia: Border of Papua and West Papua Provinces, Wondiwoi Mountains, ZMB 62384.

***Litoria viranula* Menzies, Richards et Tyler, 2008**

Papua New Guinea: Western Province, Bensbach River, SAMA R63487 (holotype), Wegamu, SAMA R63486-92 (paratypes); Indonesia: Papua Province, Merauke, SAMA R13666a-c, R13667a-d.

***Litoria wapogaensis* Richards et Iskandar, 2001**

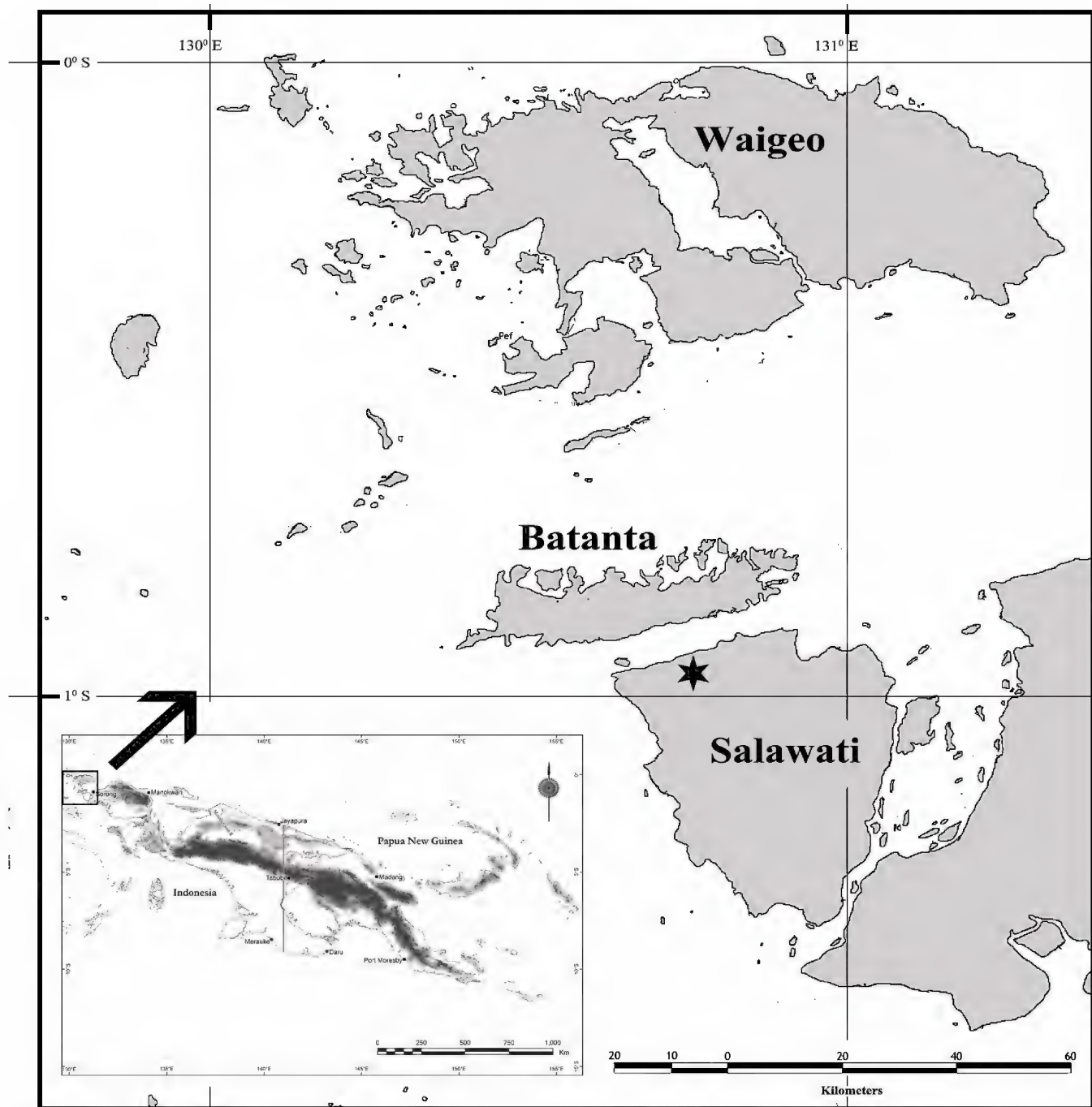
Indonesia: Papua Province, Wapoga River, MZB Amph.3873 (holotype), MZB Amph.3874-76, SAMA R54595-98 (paratypes).



Appendix 2. Tissue vouchers used.

Genus	Species	ABTC Tissue voucher	Collection Location	State/Province	Country	Specimen Voucher	GenBank Accession
<i>Callimedusa</i>	<i>tomopterna</i>	64894	-	-	-	WED55380	MT953578
<i>Hyla</i>	<i>chinensis</i>	-	-	-	-	-	AY458593
<i>Hyla</i>	<i>japonica</i>	64965	Akama, Munakata	Fukuoka	Japan	-	MT953581
<i>Litoria</i>	<i>amnicola</i> sp. nov.	90101	Salawati Island	West Papua	Indonesia	MZB Amph.12116	MW308144
<i>Litoria</i>	<i>amnicola</i> sp. nov.	90195	Salawati Island	West Papua	Indonesia	MZB Amph.12100	MT953582
<i>Litoria</i>	<i>amnicola</i> sp. nov.	90197	Salawati Island	West Papua	Indonesia	MZB Amph.12106	MW308145
<i>Litoria</i>	<i>angiana</i>	48210	Nokopo	Morobe	PNG	AMS R124118	MT953583
<i>Litoria</i>	<i>angiana</i>	48337	Nokopo	Morobe	PNG	AMS R124246	MT953584
<i>Litoria</i>	<i>arfakiana</i>	100659	Wondiwoi Mtns	Papua	Indonesia	ZMB 62116	MT953585
<i>Litoria</i>	<i>arfakiana</i>	100689	Yapen Island	Papua	Indonesia	ZMB	MT953586
<i>Litoria</i>	<i>becki</i>	116940	Mueller Range	Western	PNG	SAMA R65167	MT953587
<i>Litoria</i>	<i>becki</i>	116987	Mueller Range	Western	PNG	UPNG	MT953588
<i>Litoria</i>	<i>bicolor</i>	100072	Mitchell Plateau	Queensland	Australia	WAM R167822	MT953590
<i>Litoria</i>	<i>bicolor</i>	28366	Jabiru	Northern Territory	Australia	MAGNT R13815	MT953589
<i>Litoria</i>	<i>brevipalmata</i>	24910	Ourimbah Creek	New South Wales	Australia	-	MT953591
<i>Litoria</i>	<i>bulmeri</i>	133760	Mt. Mimi	Western	PNG	H186	MT953592
<i>Litoria</i>	<i>dorsivena</i>	112514	Wanakipe	Enga	PNG	SJR10660	MT953593
<i>Litoria</i>	<i>eucnemis</i>	32215	Peach Creek, McIlwraith Ranges	Queensland	Australia	QM J61596	MW357882
<i>Litoria</i>	<i>eucnemis</i>	90052	Foya Mountains	Papua	Indonesia	SJR6126	MW357883
<i>Litoria</i>	<i>infrafrenata</i>	15302	Cairns	Queensland	Australia	SAMA R34972	MT953596
<i>Litoria</i>	<i>infrafrenata</i>	48722	Karkar Island	Madang	PNG	AMS R124631	MT953597
<i>Litoria</i>	<i>leucova</i>	101474	Mt Stolle	Sandaun	PNG	SAMA R44092	MT953598
<i>Litoria</i>	<i>lutea group</i>	SJR1443	Bougainville	Autonomous Region of Bougainville	PNG	SJR1443	MT953599
<i>Litoria</i>	<i>micromembrana</i>	48643	Bundi	Madang	PNG	AMS R124552	MW357881
<i>Litoria</i>	<i>micromembrana</i>	98290	Abalgamut, Huon Peninsula	Morobe	PNG	SAMA R61599	MW357880
<i>Litoria</i>	<i>modica</i>	45060	Bobole	Southern Highlands	PNG	AMS R132406	MT953601
<i>Litoria</i>	<i>modica</i>	46364	Namosado	Southern Highlands	PNG	AMS R132409	MT953602
<i>Litoria</i>	<i>oenicolen</i>	118073	Baiyer River	Western Highlands	PNG	SJR15021	MT953603
<i>Litoria</i>	<i>oenicolen</i>	118074	Baiyer River	Western Highlands	PNG	SJR15022	MT953604
<i>Litoria</i>	<i>pratti</i>	142224	Yuk Creek near Tabubil	Western	PNG	SJR14541	MT953605
<i>Litoria</i>	<i>prora</i>	98252	Gobe	Southern Highlands	PNG	SJR2091	MT953607
<i>Litoria</i>	<i>prora</i>	136053	Pnyang Survey	Western	PNG	SJR14205	MT953606
<i>Litoria</i>	<i>rivicola</i>	100683	Wondiwoi Mtns	Papua	Indonesia	ZMB 67361	MT953608
<i>Litoria</i>	<i>rivicola</i>	100685	30km SE Nabire	Papua	Indonesia	ZMB 64117	MT953609
<i>Litoria</i>	<i>sauroni</i>	76032	Dark End Lumber	Gulf	PNG	SJR4989	MT953610
<i>Litoria</i>	<i>sauroni</i>	98775	Dark End Lumber	Gulf	PNG	SJR4958	MT953611
<i>Litoria</i>	<i>spartacus</i>	98336	Moro torrent	Southern Highlands	PNG	SJR2303	MT953612
<i>Nyctimystes</i>	<i>humeralis</i>	44903	Bobole	Southern Highlands	PNG	AMS R132331	MW357885
<i>Nyctimystes</i>	<i>humeralis</i>	44909	Bobole	Southern Highlands	PNG	AMS R132329	MW357884
<i>Pseudacris</i>	<i>crucifer</i>	32559	Washtenaw County	Michigan	USA	-	MT953579
<i>Pseudacris</i>	<i>crucifer</i>	32560	Washtenaw County	Michigan	USA	-	MT953580
<i>Pseudacris</i>	<i>species</i>	55395	-	-	USA	-	MT953616





Map 1. The type (and only known) locality for *Litoria amnicola* sp. nov. on Salawati Island.

